Methods: Serum biomarkers levels were measured in 18 patients with HH at time of diagnosis and after iron depletion. Iron depletion was considered to be achieved when ferritin level was below 50 microg/l. For each patient, demographic data were collected and the global articular pain (0-100 mm VAS) was assessed before and after iron depletion. Medians and ranges were reported for non-normally distributed data and means ± SD were reported for normally distributed data. Statistical analyses were performed using the Wilcoxon and the Mann-Whitney tests.
Results: All patients (10 males and 8 females, mean age 48 (11) years)) were homozygous for the C282Y mutation. Ferritin level before iron removal was 627.5 (133-3276) microg/l and duration of the iron depletion phase was 295 (70-670) days. Global joint pain (VAS) did not correlate with ferritin concentration (p=0.59) and did not decrease after iron depletion: 43±15mm vs. 36±17mm (p=0.14). Both serum levels of Coll 2-1 (marker of collagen degradation) and CPII (marker of collagen synthesis) significantly increased after iron depletion: 80.19 (55,6-113,5) nM vs. 96 (48,83-136,3) nM (p=0,03) and 731,46 (374,28-1012,37) ng/ml vs. 812,82 (535,81-1165,63) ng/ml (p=0,03), respectively. Levels of the other biomarkers were not modified by iron depletion. The severity of iron overload, as assessed by the serum ferritin level at diagnosis, was significantly correlated with the serum level of HA (marker of synovium) measured before iron depletion (r=0,60; p=0,01).
Conclusions: The results of this study suggest that i) Iron depletion in patients with genetic hemochromatosis is associated with an increased cartilage matrix turnover; ii) Synovium might play a role in the pathophysiology of hemochromatosis arthropathy.

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CHANGES IN BIOMARKER LEVELS AFTER ACUTE INJURY

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Purpose: The annual incidence of acute knee injury in the United States is estimated to be 300 cases per 100,000 population. Response to surgical treatment varies and currently there is a general lack of objective evidence to support a protective role of repair or reconstructive surgery against osteoarthritis (OA) development. Even after surgery, on average, osteoarthritis, pain, and functional impairment occur 10-20 years after severe knee joint injury in 50% of knee injury patients. We hypothesize that after joint injury, such as trauma or anterior cruciate ligament damage, the inflammatory cytokines produced lead to irreversible damage to the cartilage and possibly the bone microstructure resulting in reduced joint functionality and eventual early onset OA. Here we investigate the changes in biomarkers in the synovial fluid (SF) of a small intra-articular IL-1Ra intervention study (n=11) at time of injury (baseline) and one month later (follow-up) when the patient underwent reconstructive surgery.
Methods: SFs were collected under IRB approval at baseline and at follow-up from an IL-1Ra intervention study of 11 patients: after initial SF collection, 6 patients were treated with a single dose of 150 mg IL-1Ra (Anakinra) and 5 placebo patients were treated with saline. Changes in biomarkers (see Table) were determined using ELISA assays and all data were analyzed using Wilcoxon signed rank test.
Results: We investigated a wide range of biomarkers in SF, see table. There were significant increases in the SF levels of collagen type I/II related markers (CTxI, NtxI, CtxII, and C1,2C) from baseline to follow-up. In contrast, SF proteoglycan loss was elevated after acute injury then decreased significantly by one month as represented by GAG. SF markers of protein age indicative of the turnover state (racemized D-Asp/protein and D-Ser/protein) increased significantly from baseline to follow-up.

	Mean (SD)		
Analyte	Baseline	Follow-up	P value
Collagen Biomarkers			
C2C (ng/ml)	180.3 (37.4)	208.9 (56)	P=0.3
CtxII (µg/ml)	**0.56 (0.56)**	**1.09 (0.63)**	**P=0.01**
CPII (ng/ml)	301.9 (243.4)	318.3 (185)	P=0.8
C1,2C (µg/ml)	**0.26 (0.11)**	**0.37 (0.07)**	**P=0.039**
CtxI (ng/ml)	**0.73 (0.37)**	**1.22 (0.67)**	**P=0.004**
NtxI (nM BCE)	**15.3(4.2)**	**19.6 (7.8)**	**P=0.008**
GAG/Proteoglycan Biomarkers			
FA846 (ng/ml)	1131(150)	886.5 (295.7)	P=0.07
HA (mg/ml)	660 (468.8)	706.6 (344.9)	P=0.9
GAG (mg/ml)	**191.2 (135.2)**	**91.8 (77.3)**	**P=0.0098**
Other Biomarkers			
sCD44 (ng/ml)	127.8 (46.3)	105 (28.2)	P=0.3
Cathepsin K (pM/L)	20.7 (16.7)	19.7 (13.8)	P=0.5
Osteocalcin (ng/ml)	14.8 (5.1)	17.1 (6.2)	P=0.3
Total protein (mg/ml)	**63.4 (23.3)**	**50.9 (27.7)**	**P=0.002**
COMP (mg/ml)	**128.1 (78.0)**	**81.0 (49.4)**	**P=0.027**
Post-translational protein modification biomarkers			
D-Asp/protein mM/mg	**0.26 (0.04)**	**0.31 (0.08)**	**P=0.0039**
D-Ser/protein mM/mg	**0.03 (0.01)**	**0.05 (0.02)**	**P=0.05**
PIMT/protein units/mg	0.011 (0.007)	0.011 (0.006)	P=0.8

Table summarizing the synovial fluid and serum biomarker data. N=10 paired samples for the synovial fluid.

Conclusions: We investigated a wide range of biomarkers in the SF of acute injury patients to better understand the effects of the early inflammatory events upon joint damage after acute injury. We observed significant early proteoglycan loss which decreased with time, and an increase in collagen loss. This pattern of initial proteoglycan loss followed by collagen loss is characteristic of pro-inflammatory induced cartilage damage. Markers of protein-age compatible with degeneration of older and deeper cartilage macromolecules were also elevated. These data suggest that there is significant cartilage and bone damage during the initial weeks after acute injury which left untreated could enhance the risk of later OA development. These data also suggest that specifically targeting the early inflammatory events after acute injury would have potential long term benefits.

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EVALUATION OF AN AGGRECAN BIOMARKER FOR ASSESSMENT OF AGGRECANASE INHIBITOR ACTIVITY

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Purpose: This study focuses on the development of an drug activity biomarker of aggrecanase activity in osteoarthritis (OA). Degradation of cartilage matrix proteins, such as aggrecan, is one of the mechanisms that leads to cartilage dysfunction. Attempts to monitor this degradation require a well-validated biomarker assay, especially to decrease lengthy OA clinical trials. This study centers on a biomarker which will respond in a dose-dependent manner to small molecule aggrecanase inhibitors in different biological models. The biomarker centers on the aggrecan degradation (peptide fragment ARGSVIL) resulting from aggrecanase cleavage at the 373Glu - 374Ala bond in the interglobular domain of aggrecan. Quantification of levels of this biomarker could allow potential assessment of patient differentials, such as inter- and intra-variability in patient populations, efficiency of drug inhibition, and a potential for diagnostic/prognostic capabilities or patient stratification/selection for tailored therapeutics.
Methods: Here, we analyze biological samples from bovine and human articular cartilage explants and human OA urine to assist in understanding the values of the ARGSVIL biomarker in these tissues, inter- and intra-patient variability, and the ability to inhibit aggrecan degradation via use of inhibitor compounds. The analysis of these biological samples is performed using a novel immunoaffinity LC/MS/MS assay or a recently developed ELISA assay based on an improved ARGS neoepitope antibody (BC3-C2) that was developed to measure levels of the ARGSVIL peptide biomarker.

Results: Aggrecan degradation and its inhibition demonstrated cross-correlation between bovine articular cartilage explants assayed via DMMB proteoglycan assay and IA/LC/MS/MS assays. Dose-dependent reduction of the ARGS biomarker was also demonstrated in human articular cartilage explants. The ARGS biomarker was further detected in human urine analyzed by IA/LC/MS/MS with a high degree of variability between patients. Due to the development of an improved ARGS neoepitope antibody, an BC3-C2/HABR ELISA was utilized to analysis human articular cartilage explant samples and clinical patient urine samples. Approximately 500 pg/mL biomarker values were detected in the ELISA, agreeing with data obtained from the IA/LC/MS/MS data and literature reports.
Conclusions: The ARGSVIL peptide demonstrates high potential as a drug activity biomarker of aggrecan degradation. Current results validate its presence in human urine and demonstrate dose response reduction with aggrecanase inhibition. This biomarker promises to speed dose selection and predict target inhibition in human clinical studies of aggrecanase inhibitors.

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DIURNAL VARIATION OF AMINO ACIDS AND ACYLCARNITINES IN A COHORT WITH KNEE OA

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Purpose: Biomarker levels can fluctuate in response to daily activity, including feeding. In order to ascertain the degree and direction of these changes, and in anticipation of investigating the interaction of these agents with OA, markers of energy metabolism were measured at various time points, including before and after a mixed-nutrient meal.
Methods: 40 subjects with radiographic OA of at least one knee were admitted overnight to the General Clinical Research Center of Duke University to undergo serial serum sampling. Blood was collected in the evening (T=3, 6-8pm, n=40), before rising from bed or eating (T=0, 8am, n=40), 1 hour after rising but before eating (T=1A, 9 am, n=20), 1-2 hours after rising and breakfast (T=1, 9-10 am, n=40), and at noon (T=2, n=20). Breakfast included protein, carbohydrate, and fat. Quantitative mass spectrometry of 15 amino acids (AAs), and 45 acyl carnitines (ACs), was performed by stable-isotope dilution. The mean of the values for each time point was normalized to the mean at baseline (T=0). Values for acyl carnitines were log-transformed.
Results: All 15 amino acids displayed significant ($p<0.05$) changes between baseline and various time points ranging from 4-43% (Table 1). The most notable increase occurred after consumption of a mixed meal, when 14 AAs increased 7-23%. Statistically significant increases continued throughout the day for most AAs in a monotonic pattern. Histidine showed the greatest stability, with a statistically significant change occurring only at T3 compared to baseline (T0). Alanine, proline, valine, leucine/isoleucine, methionine, phenylalanine, tyrosine, Aspartate/asparagine, ornithine, and arginine varied significantly in at least three of three of the timepoints relative to baseline.
All 45 acylcarnitines (ACs) displayed statistically significant changes at various time points as compared to baseline (15 representative ACs shown in Table 2). Unlike AAs, which generally displayed the same monotonic pattern to varying degrees, the ACs varied widely in their diurnal profiles. The ACs also generally displayed smaller increases (typically 1-10%) as compared to AAs (4-43%). Similar to AAs, an increase in serum concentration was most common after ingestion of a mixed meal, as 31 ACs displayed statistically significant increases in the range of 1-16%.
Conclusions: From this preliminary study it is evident that major classes of energy metabolites, including amino acids and acyl carnitines, vary significantly in response to normal daily activities including food intake. Elucidating these diurnal variations in metabolites is an important early step in understanding the greater complexities of energy balance. Furthermore, we hypothesize that many of the agents of energy balance are intricately involved with biomarkers of osteoarthritis, and defining underlying metabolic profiles will promote a more complete understanding of health and disease states. Finally, of significant practical importance, this study underscores the need for researchers to plan deliberately and act consistently when collecting samples for biomarker measurement, since inconsistent sampling could introduce spurious variations in the data.

Table 1 Amino Acid levels normalized to baseline (T0)

	T1A Fasting Active 1 hr		T1 Fed Active 1-2 hr		T2 Fed Active 4hr		T# Fed Active 12 hr	
	Median	P value	Median	P value	Median	P value	Median	P value
Gly	1.00	1.00	**1.07**	**0.0001**	1.04	0.294	**1.07**	**0.018**
Ala	1.02	0.0973	**1.23**	**<0.0001**	**1.07**	**0.030**	**1.18**	**<0.0001**
Ser	1.06	0.189	**1.07**	**<0.0001**	1.01	0.648	1.03	0.1822
Pro	**0.96**	**0.044**	**1.15**	**<0.0001**	**1.15**	**0.0003**	**1.37**	**<0.0001**
Val	1.00	0.956	**1.11**	**0.0002**	**1.10**	**0..0037**	**1.28**	**<0.0001**
Leu/Iso	1.02	0.841	**1.17**	**<0.0001**	**1.12**	**0.024**	**1.39**	**<0.0001**
Met	1.01	0.123	**1.21**	**<0.0001**	**1.18**	**0.0121**	**1.43**	**<0.0001**
His	0.99	0.330	0.99	0.1046	1.00	0.985	**1.05**	**0.018**
Phe	**1.04**	**0.048**	**1.16**	**<0.0001**	**1.11**	**0.0006**	**1.27**	**<0.0001**
Tyr	**1.04**	**0.008**	**1.14**	**<0.0001**	**1.18**	**0.0037**	**1.28**	**<0.0001**
Asp/Asn	**1.15**	**0.0006**	**1.26**	**<0.0001**	1.13	0.0897	**1.22**	**<0.0001**
Glu/Gln	**1.09**	**0.006**	**1.12**	**<0.0001**	1.05	0.1429	1.01	0.2056
Orn	0.99	0.7562	**1.12**	**0.0033**	**1.16**	**0.0042**	**1.25**	**<0.0001**
Cit	1.01	0.349	**0.92**	**0.0077**	0.99	0.3884	0.95	0.7216
Arg	**1.09**	**0.0009**	**1.20**	**<0.0001**	1.08	0.240	**1.21**	**<0.0001**

Table 2 Acylcarnitine levels normalized to baseline

	T1A Fasting Active 1hr		T1 Fed Active 1-2 hr		T2 Fed Active 4hr		T# Fed Active 12 hr	
	Median	P value	Median	P value	Median	P value	Median	P value
Acetyl carnitine	**1.07**	**0.002**	**1.06**	**<0.0001**	**1.05**	**0.0266**	1.0147	0.8742
Propionyl carnitine	**1.07**	**0.0064**	**1.08**	**0.0003**	**1.12**	**0.0009**	**1.14**	**<0.0001**
Succinyl carnitine	1.00	0.18	**0.01**	**0.008**	**1.017**	**0.024**	**1.02**	**<0.0001**
Octanoyl carnitine	**1.17**	**<0.0001**	**1.08**	**<0.0001**	**1.04**	**0.02**	0.97	0.26
Adipoyl carnitine	**1.01**	**0.0009**	**1.01**	**0.0219**	**1.01**	**0.0441**	1.00	0.72
Decatrienoyl carnitine	**1.02**	**0.0037**	**1.02**	**0.0097**	**1.04**	**0.0003**	**1.02**	**<0.0001**
Decadienoyl carnitine	**1.01**	**0.0014**	**1.02**	**<0.0001**	**1.03**	**0.0032**	**1.01**	**<0.0001**
Decenoyl carnitine	**1.18**	**<0.0001**	**1.05**	**<0.0001**	**1.04**	**<0.0001**	1.00	0.41
Decanoyl carnitine	**1.21**	**<0.0001**	**1.07**	**<0.0001**	**1.04**	**0.0049**	**0.97**	**0.0125**
Suberoyl carnitine	**1.02**	**0.0001**	**1.02**	**<0.0001**	**1.02**	**0.0056**	1.00	0.8535
Dodecenoyl carnitine	**1.10**	**<0.0001**	**1.04**	**<0.0001**	**1.04**	**0.0017**	**0.98**	**0.02**
Lauroyl carnitine	**1.07**	**<0.0001**	**1.04**	**<0.0001**	**1.04**	**0.0003**	1.01	0.40
Hexenedioyl carnitine	**1.01**	**0.0094**	**1.01**	**0.0255**	**1.02**	**0.0064**	**1.01**	**0.0275**
Octenedioyl carnitine	**1.02**	**0.0006**	**1.01**	**<0.0001**	**1.02**	**0.002**	1.01	0.145
Palmitoleoyl carnitine	**1.02**	**<0.0001**	**1.01**	**0.0003**	1.00	0.99	**0.99**	**0.0023**

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CYTOKINE-INDUCED AGGRECANASE- AND MMP-DERIVED RELEASE OF AGGRECAN FRAGMENTS INTO THE SUPERNATANTS OF HUMAN OA ARTICULAR CARTILAGE CULTURES; DIFFERENCES TO CORRESPONDING PROFILES ORIGINATING FROM BOVINE CULTURES

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Purpose: Quantitative measurement of aggrecan fragments released from explants cultures is a validated model for the study of metabolic processes relevant during cartilage degradation. We wanted to investigate, if a new series of neo-epitope specific immunoassays could be applied in the profiling of aggrecanase- and MMP-derived aggrecan fragments released into the supernatant of articular cartilage explants originating from patients with osteoarthritis.